PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Matilde **BUSTOS DE ABAJO**, et al

Serial No.: 10/798,219 Group No. 1633

Filed: MARCH 11, 2004 Examiner: A.M.S. Wehbe

Confirmation No.: 3487

For: USE OF CARDIOTROPHIN IN LIVER DISEASES

Attorney Docket No.: **U 015070-8**

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

SUBSTITUTE DECLARATION UNDER CFR 1.132

Kindly substitute the attached Declaration Under CFR 1.132 with original ink signature for the document previously filed on September 10, 2009.

Respectfully submitted,

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CERTIFICATE OF MAILING/TRANSMISSION (37 CFR 1.8a)

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DECLARATION UNDER 37 CFR 1.132

I, Matilde BUSTOS DE ABAJO, declare and say as follows:

1. I am a co-inventor of the invention described and claimed in US Patent Application Serial No. 10/798,219 ("the application"). I make this declaration in support of the application. My curriculum vitae is annexed hereto as Exhibit 1.

2. I believe that, as of the application filing date, a person of skill in the art to which the application pertains would have had an advanced degree in hepatology or the like and/or at least 5 years of experience working in this area. Such person would have knowledge of the publications discussed below.

3. The invention described and claimed in the application is based at least in part upon our discovery that the administration of cardiotrophin-1 (CT-1) to a subject whose liver has suffered injury (as occurs, e.g., when functional liver mass is diminished or the liver

is resected) can provide a therapeutic effect to the subject. In particular, we found, unexpectedly, that CT-1 not only prevents liver damage in healthy cells but it has also anti-apoptotic activity in already injured livers resulting in a marked therapeutic effect.

4. To explain, in an injured liver, several hepatocyte populations might co-exist, depending on its level of damage: dead hepatocytes, damaged hepatocytes and non-affected (healthy) hepatocytes. We found that CT-1 has regenerative activity in such injured livers inducing proliferation of surviving hepatocytes that can replace the dead ones. Equally and additionally crucial, CT-1 has antiapoptotic activity that prevents death of injured hepatocytes and healthy hepatocytes in the presence of an agent toxic for the liver. This cytoprotective activity increases the pool of surviving hepatocytes when the liver is injured. This facilitates the regenerative response of hepatocytes that will restore the liver functional mass.

5. I understand that the examiner of the application has cited the following publications to show that, as of the filing date of the application, one of skill in the art would have had a reasonable expectation of success in the use of CT-1 to treat a subject with an injured liver that has suffered a functional loss of liver cells: Jin et al, (1996) Cytokine, Vol. 8 (12) 920-926; Costa et al, U.S. Patent Application Publication 2002/0187936 and Hogaboam et al, U.S. Patent 6,719,969. In particular, I understand that the Examiner contends that: (a) Jin et al's disclosure that CT-1 induces liver growth *in vivo* demonstrates that CT-1 can stimulate hepatocyte proliferation and/or differentiation; and (b) the disclosure in Costa and Hogaboam that **different** proteins (i.e., proteins other than CT-1) that induce hepatocyte proliferation may be useful to treat subjects with liver damage provides a reasonable expectation that CT-1 would also be useful to treat subjects with liver damage. I respectfully disagree with these contentions.

6. With respect to the examiner's contention (a), Jin et al. do not provide any data or evidence showing hepatocyte replication or proliferation after CT-1 administration, but just an increase of the liver weight. No further comments or suggestions about the meaning of such observation are provided. It should be considered that effects other than hepatocyte proliferation can account for the increase in liver weight in the mice in the Jin et al study. Thus, one of skill in the art could not have concluded that the increased liver

weight in the Jin et al. study was due to hepatocyte proliferation. For example, hepatomegaly (that is increased liver weight) can be observed in several situations such as steatosis, toxic hepatitis, cholestasis, acute liver failure, congestive heart failure (right ventricular failure). On the other hand, different factors can increase the weight of healthy livers by increasing the size of liver cells without increasing the number of hepatocytes (that is, without stimulating the replication of liver cells). For instance, dexamethasone is able to induce hypertrophy of the hepatocytes increasing the liver weight; while this drug induces a severe inhibition of DNA synthesis. In this case, liver hypertrophy is transient, because after dexametaxone withdrawal, hepatocyte size is re-established within a few days. (Nagy P, Teramoto T, Factor VM, Sánchez A, Schnur J, Paku S, Thorgeirsson SS. Reconstitution of liver mass via cellular hypertrophy in the rat. *Hepatology,* 2001 Feb; 33(2):339-345).

7. Indeed, experimentation that I performed or that was performed under my supervision and control shows that the increased weight described in Jin et al was likely **not** due to such proliferation. Specifically, we have administered chronic doses of CT-1 to healthy mice, as Jin et al. have done (Jin H, Yang R, Keller GA, Ryan A, Ko A, Finkle D, Swanson TA, Li W, Pennica D, Wood WAI, Panoni NF. In vivo effects of cardiotrophin-1. *Cytokine.* 1996; 8 (12): 920-926) to confirm data by these authors and to investigate the mechanism of increased liver weight when CT-1 was given to mice with normal livers. As controls we used mice given saline (vehicle) instead of CT-1. At the end of the experiment, animals were sacrificed and the livers were analyzed. Liver weight was increased in rCT-1-treated animals compared to saline-treated mice (1.112 ± 0.02 *versus* 0.977 ± 0.03). The histopathological study of the liver of CT-1-treated mice showed no mitosis and the immunohistochemistry study for Ki-67 was negative (Figure 1A). Ki67 is a nuclear protein that is expressed in proliferating cells. Ki-67 has been used as a marker for cell proliferation. Furthermore, the expression of cyclin D1 in the livers from rCT-1 treated animals was negative as well as in the saline-treated animals (Figure 1B). The expression of cyclin D1 in the liver regulates transition from G1 to S phase in cell cycle and is used as a marker for cell proliferation.

The Figure 1 clearly shows that CT-1 treatment to healthy mouse does not induce hepatocyte proliferation.

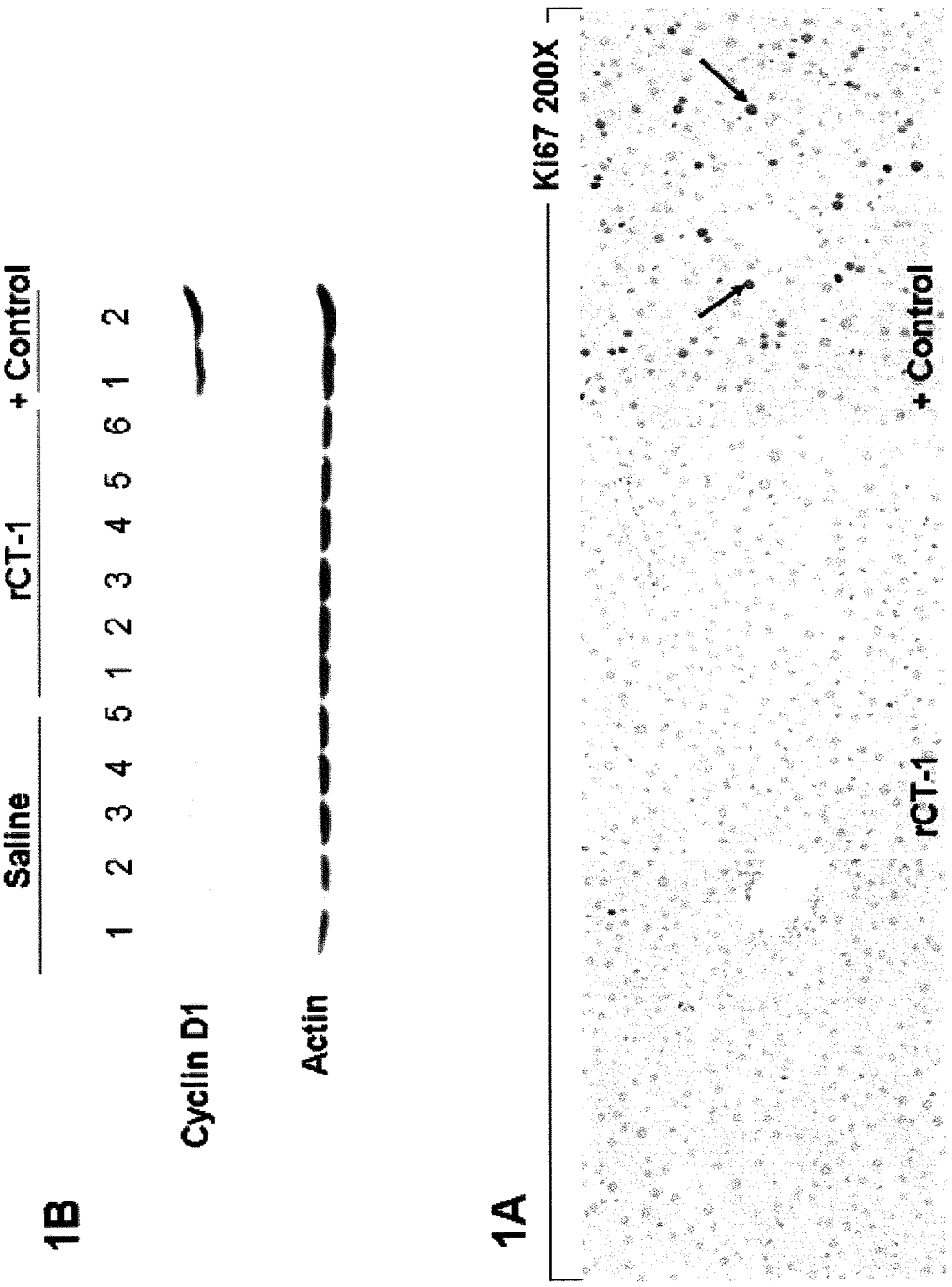

Figure 1:

Saline: Livers from saline-treated animals

rCT-1: Livers from rCT-1-treated animals

Positive control (+ Control): To show that the technique is reliable we employed a positive samples obtained from mouse livers after partial hepatectomy (proliferation of hepatocytes)

The above data indicate that, in spite of the increased liver weight, we could not see any hepatocyte replication. I accordingly believe that the increased liver weight observed by chronic CT-1 treatment is not due to hepatocyte replication.

8. With respect to the examiner's contention (b), I respectfully call attention to other publications that show why one of skill in the art would not expect that any and all substances with hepatocyte proliferative effects could be effectively administered to patients suffering from liver damage. By way of example, Bockhorn et al. (Bockhorn M, Schóllmann S, Optiz B, Sotiropoulos GC, Sheu SY, Niehaus E, trippler M, Frilling A, Broelsch CE, Schlaak JF. Vascular endothelial growth factor does not improve liver regeneration and survival after 90% subtotal liver resection. *Hepatol Res.* 2007 May;37(5):353-9) show that, although VEGF is a well known growth factor, the administration does not improve liver regeneration and survival after 90% subtotal liver resection. As another example, Klemm et al. (Klemm K, Eipel C, Cantré D, Abshagen K, Menger MD, Vollmar B. Múltiple doses of erythtropoietin impair liver regeneration by increasing TNF-alpha, the Bax to Bcl-xL ratio and apoptotic cell death. *PloS One* 2008;3(12):e3924. Epub 2008 Dec 11.) observe that, although erythroopoietin has been recognized as an antiapoptotic, mitogenic and tissue-protective cytokine, múltiple doses of erythropoietin impaired liver regeneration by increasing apoptotic cell death (Klemm K, Eipel C, Cantré D, Abshagen K, Menger MD, Vollmar B. Múltiple doses of erythtropoietin impair liver regeneration by increasing TNF-alpha, the Bax to Bcl-xL ratio and apoptotic cell death. *PloS One* 2008;3(12):e3924. Epub 2008 Dec 11).

9. In view of the above considerations, I respectfully submit that the publications cited by the examiner would not have provided one of skill in the art with even a reasonable expectation of success in the use of CT-1 for the treatment of liver damage in a subject.

10. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity

of the application or any patent issued thereon.

Date: 09 / 09 / 2009

Name: Matilde BUSTOS DE ABAJO